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Abstract

In the UK, there has long been a need for consuming foods high in vitamin D in order to prevent diseases associated with low bone mineral density such as osteoporosis in adults and rickets in children. It has been claimed that potentially, 50% of the UK adult population are vitamin D insufficient in winter and spring time, actual deficiency may be 16%. Those workers who commence their working hours in the evening may be deprived of vitamin D synthesising UVB. Moreover, the physical maladaption to altered circadian rhythms experienced by many shift and, particularly,night workers has been identified as a leading cause of change to dietary intake. The previous literature has documented associations between nocturnal working schedules and adverse health effects. The influence of working place shift schedules i.e. night and day shifts, on vitamin D status has not been researched extensively.

Introduction

The importance of a balanced diet has been highlighted and publicised in the UK as the main message for the prevention of numerous diseases by the National Health Service (NHS, 2013). The NHS advocates a variety of foods such as, fruits and vegetables, starchy foods, meat, fish and eggs and dairy foods, and correct portion control to achieve a healthy body weight i.e. BMI 20-25 kg/m^2 (NHS, 2013). As well as a 'healthy body weight', a balanced diet should also provide adequate nutritional value, which can be measured by dietary references values outlined by the Scientific Advisory Committee on Nutrition (SCAN, 2011). However, SACN and the National Institute of Clinical Excellence (NICE, 2013) do not provide dietary reference values for vitamin D for men and non-pregnant women aged 4-65 due to ambiguity regarding toxicity and a threshold for adequacy and deficiency. Nevertheless, the general consensus from the existing research suggests a large proportion of the UK population are vitamin D deficient (Cancer Research UK, 2010).

Vitamin D

Vitamin D is an essential fat-soluble nutrient which is crucial to calcium homeostasis, thus bone mineral density, as well as other roles within the body such as immune function (Cancer Research UK, 2010).

Endogenous and Exogenous sources

Vitamin D_3 is synthesised endogenously when the epidermis layer of the skin is exposed to ultraviolet blue (UVB) radiation at a latitude-dependent intensity, 290-315 nm wavelength (Holick, 2008). The UVB radiation converts 7-dehydrocholesterol, causing a break between carbons 9 and 10, into previtamin D_3. Previtamin D_3 can be identified in two forms, cis, cis or cis, trans. The cis, cis form of previtamin D_3 rapidly converts to vitamin D_3, whereas the cis, trans form takes over 24 hours to convert by 50% (Holick, 2008). To prevent hypervitaminosis D, excess previtamin D_3 is degraded by UVB exposure. The humans can consume, plant derived, vitamin D_2 and, animal derived, vitamin D_3 from dietary intake. Vitamin D_3 and D_2 are transported via chylomicrons to adipose tissue where it is stored or released attached to vitamin D binding protein, depending on demand

Metabolism

The liver's Vitamin D-25-hydroxylase converts vitamin D_3 to 25-hydroxyvitamin D (25 [OH] D). The subsequent metabolite is inactive until renal secretion of 25 (OH) D-1α-hydroxylase, up-regulated by parathyroid hormone (PTH), fibroblast growth factor and others, converts

25 (OH) D to metabolically active 1, 25-dihydroxyvitamin D (Holick, 2008). 1, 25-dihydroxyvitamin D has a half-life of 3-6 hours, whereas, 25 (OH) D has a half-life of 2-3 weeks which therefore gives a more accurate indication of vitamin D status.

Mechanism of action

The primary action of 1, 25-dihydroxyvitamin D (1, 25 [OH] D) is to promote calcium absorption in the intestine via up-regulation of epithelial calcium channel (Anderson, turner & Morris, 2012). 1, 25 (OH) D enters the intestinal cell and binds to the vitamin D receptor (VDR), also found on the kidney and bone, which enables transportation to the cell nucleus and production of calbindin mRNA. Calbindin facilitates calcium absorption at the intestinal brush border membrane. The VDR is also found on the kidney and bone, enabling 1, 25 (OH) D to up-regulating receptor activator of nuclear factor kappa-B ligand to increase osteoclastic bone resorption (Anderson, Turner & Morris, 2012). 1, 25 (OH) D also provides negative feedback which regulates the renal secretion of 25-hydroxvitamin D-1α-hydroxylase and provides negative feedback to the parathyroid gland to down-regulate PTH secretion.

Dietary Sources

In the UK, there has long been a need for consuming foods high in vitamin D in order to prevent diseases associated with low bone mineral density such as osteoporosis in adults and rickets in children. Indeed prior to the discovery of vitamin D, cod liver oil was used to prevent rickets in UK children during the 20th century (Pettifor, 2004). There are very few natural sources of vitamin D_2. Mushrooms which contain vitamin D_2 (ergosterol), a precursor to ergocalciferol, which when exposed to UVB can increase humans' vitamin D_2 content (United States Department of Agriculture, 2009). Vitamin D_3, is found in few dietary sources (Table 1), the most potent source of which is oily fish, such as tuna, salmon, herring, mackerel and sardines (Biesalski, 2011).

Table 1: Commonly found dietary sources of vitamin D (adapted from Calvo, Whiting & Barton, 2004).

Source of vitamin D	Amount (grams)	Vitamin D content (Micrograms)
Herring	85g	34.5 μg
Swordfish, cooked	85g	14.15 μg
Pink salmon, canned	85g	13.25 μg
Mackerel	85g	7.65 μg
Tuna, canned	85g	5 μg
Quaker Nutrition for Women Instant Oatmeal	1 packet	3.85 μg
Pork, spareribs, cooked	85g	2.2 μg
Fortified Yogurt	170g	2.2 μg
Fortified Margarine	1 tablespoon	1.5 μg
Liver, Beef cooked	99g	1.225 μg
Egg (vitamin D in yolk)	1 large	1.025 μg
Cheese, Swiss	28g	0.15 μg

There are a number of foods which have been fortified in the USA, such as milk, milk products and cereals (Calvo, Whiting & Barton, 2004). Indeed, the maximal level of vitamin

D fortification for fluid, acidified and concentrated milk is 42 IU (1.05 µg) per 100g (Calvo et al., 2004). Margarine and calcium-fortified fruit juices and drinks can be optionally fortified up to 8.3 µg /100g and 100IU/amount customarily consumed (Calvo et al., 2004). Whereas, in the UK the fortification of margarine with 800-1000µg of vitamin A and 7.05-8.82µg of vitamin D is mandatory (Food Standards Agency, 2010). However, common practice involves fortification using cholecalciferol, an animal derived compound, excluding non-meat eaters i.e. vegans and vegetarians consumers (SACN, 2007). There have been a number of UK studies which have reported estimate vitamin D intakes (Table 2).

Table 2: Dietary intakes of vitamin D and calcium from previous studies

Study	Participants (n) Age (range, years)	Location	Dietary Assessment method	Medication	Dietary intakes		Supplementation
					Vitamin D	Calcium	
New, Bolton-Smith, Grubb & Reid (1997)	N = 994 Women (44-50 yrs)	Aberdeen, Scotland	Food Frequency Questionnaire	Not Assessed	3.5 ± 2.3 µg/d*	1060 ± 344 mg/d*	Not Assessed
New et al. (2000)	N = 62 Women (45-55 yrs)	Aberdeen, Scotland	Food Frequency Questionnaire	Not Assessed	3.41 ± 2.51 µg/d*	1101 ± 377 mg/d*	Not Assessed
Macdonald, New, Golden, Campbell and Reid (2004)	N = 891 Women (44.9-53.5 yrs)	Aberdeen, Scotland	Food Frequency Questionnaire	Not Assessed	4.1 ± 2.4 µg/d*	1032 ± 315 mg/d*	Vitamin D = 5.5 ±3.8 µg/d* Calcium = 1061 ± 333 mg/d*
Macdonald, New & Reid (2005)	N = 898 Women (45-54 yrs)	Aberdeen, Scotland	Food Frequency Questionnaire	Not Assessed	3.20 ± 2.07 µg/d*	1052 ± 327 mg/d*	Vitamin D (n = 120) = 4.4 ± 2.2 µg/d* Calcium (n = 43) = 325 ± 277 mg/d*
Macdonald, Mavroeidi, Barr, Black, Fraser & Reid (2008)	N = 2598 Women (44.2-56.3 yrs)	Aberdeen, Scotland	Food Frequency Questionnaire	40.2% never used HRT 22% used HRT 37.8% use HRT	4.2 ± 2.5 µg/d*	1057 ± 334 mg/d*	Vitamin D = 5.8 ± 4.0 µg/d* Calcium = 1112 ± 382 mg/d*

Crowe, Steur, Allen, Appleby, Travis & Key (2010)	ME = 1359 (Females = 79%) 55 ± 10.0 yrs* FE = 208 (Females 86%) 51 ± 12.0 yrs* Veg = 417 (Females = 78%) 48 ± 13.0 yrs* V = 87 (Females = 61%) 44 ± 14.0 yrs*	Oxford, UK	Food Frequency Questionnaire	ME = 23% used HRT FE = 22% used HRT Veg = 19% used HRT V = 17% used HRT	ME = 3.1 95% CI 3.0, 3.2 µg/d** FE = 2.2 95% CI 2.1, 2.4 µg/d** Veg = 1.2 95% CI 1.1, 1.3 µg/d** V = 0.7 95% CI 0.6, 0.8 µg/d**	ME = 1026 ± 311 mg/d* FE = 1019 ± 353 mg/d* Veg = 1019 ± 379 mg/d* V = 557 ± 188 mg/d*	ME = 55% said yes FE = 73% said yes Veg = 57% said yes V = 51% said yes

*Data presented as Mean ± Standard Deviation
** Data presented as geometric mean and 95% Confidence Intervals
HRT = Hormone Replacement Therapy
ME = Meat Eaters
FE = Eaters
Veg = Vegetarians
V = Veg

The studies presented in Table 2 indicate an average dietary intake of vitamin D of 3.6 – 4.0 μg/d and 1050 mg/d for calcium. The dietary intakes were augmented >4.5 μg/d, and by 300-1100 mg/d, with supplementation. The study by Crowe et al. (2010) highlights the decreased number of vitamin D rich dietary sources available to vegetarians and vegans, such dietary beliefs are not recorded in the other studies. Furthermore, these studies are limited to a specific geographical area i.e. Aberdeen, Scotland. Indeed, the vitamin D_2 i.e. mushrooms, and D_3 i.e. oily fish, content of Scottish foods may be higher or lower than foods consumed in the Crowe et al. study. Similarly, the FFQ used in the studies may not have been sensitive enough to detect the different consumptions of farmed fish and wild caught fish.

The studies in Table 2 have used a postmenopausal study cohort typically aged 44-55, the dietary intakes of which may not be representative of a younger premenopausal cohort. The wealth of research (Macdonald et al., 2004; Macdonald, New & Reid) and health advice may influence populations, particularly postmenopausal women, to consciously consume more vitamin D and calcium rich foods to counter the loss of bone mineral density. Whereas a premenopausal cohort may not consciously consume more vitamin D and calcium foods. Additionally all of the studies in Table 2 have female dominated cohorts; males may have had an increased or decreased intake of vitamin D rich dietary sources. Indeed, the study by Crowe et al. (2010), reported significantly ($p < 0.001$) lower vitamin D intakes by vegetarians and vegans when compared to meat eaters, however, did not report the gender differences in intakes. None of the studies in Table 2 examined whether different working schedules influence the dietary intake of vitamin D and calcium.

Vitamin D deficiency

It has been claimed that 50% of the UK adult population are vitamin D insufficient in winter and spring time, actual deficiency may be 16% (Cancer Research UK, 2010). The level of vitamin D adequacy has been questioned in recent research due to the role of vitamin D in calcium homeostasis. The Institute of Medicine (2010; the Endocrine Society, 2011) identify deficiency when 25 (OH) D concentrations fall <50 nmol/L, whereas, the Wandsworth National Health Service Guidelines (2010) and Canadian Paediatric Society (2007) identify concentrations <25 nmol/L as deficient. Therefore, due to the current ambiguity regarding adequate concentrations, there is no recommended daily intake of vitamin D for men and women (when not pregnant or lactating) aged 4-64 (DoH, 2013).

There are number of factors which contribute to insufficiency and deficiency in the UK such as, minimal skin exposure due to extensive clothing, large built up areas preventing direct sunlight, long working hours relative to other countries, pregnancy, obesity, cloud cover, age and ethnicity. The aging population are more vulnerable to vitamin D deficiency as their ability to endogenously produce vitamin D due to a diminished presence of 7-dehydroxycholesterol on the epidermis, as well as a tendency to become less active (Zhang & Naughton, 2010). However, data from 13,183 vitamin D assessments from Tower Hamlets, London, found 45% of Caucasian, Black and South Asian people aged <16 yrs had serum 25 (OH) D levels <25 nmol/L, whereas, only 26% of people >64 yrs had levels <25nmol/L (Barts and the London Clinical Effectiveness Group, 2011). However, there may have been more Asian people in the <16 yr category than in the >64yr category. Indeed, the study reported 42% of Asian (*n* = 8361) people had serum 25 (OH) D levels <25 nmol/L, whereas, only 17% of Caucasian (*n* = 2630) participants had levels <25 nmol/L. People with heavily pigmented skin have increased UVB absorbing melanin, which research suggests, provides African Americans' with an innate Sun Protection Factor 15 (Holick & Chen, 2008). It has been reported that people with heavily pigmented skin may require up to 10-50 times more UVB exposure to achieve similar vitamin D synthesis. Those from a South Asian and Afro-Caribbean descent are at the greatest risk of vitamin D deficiency, particularly at northern latitudes, such as Canada, UK and Scandinavia where there is little UVB radiation (SACN, 2003). The religious beliefs of some in the UK requires them to cover the vast majority of their skin with clothes, preventing the UVB irradiation of 7-dehydroxycholesterol (Barts and the London Clinical Effectiveness Group, 2011).

There has been significant research assessing the adequacy of different populations in terms of vitamin D status (Table 3). However, due to the confounding factors associated with 25 (OH) D concentrations such as UVB exposure, age and dietary intake, based on the existing data, it is difficult to draw conclusions representative of specific populations.

Table 3: Studies exploring vitamin D levels in various populations

Source (publication date)	Number of Participants (n=)	Age Range *	Ethnicity	Location	25 hydroxyvitamin D (nmol/L)*	
Lowe, Guy, Mansi, Peckitt, Bliss, Wilson & Colston (2005)	Control group n = 131 Case (Breast Cancer) group n = 179	Control group = mean = 58, range = 36-80 yrs Case group = mean = 58, range 34-84 yrs	Caucasian	St. George's Hospital, London, UK	Cases <50 nmol/L n = 54, 30% 50-100 nmol/L n = 69, 39% 100-150 nmol/L n = 43, 24% >150 nmol/L n = 13, 7%	Control <50 nmol/L N = 21, 12% 50-100 nmol/L n = 79, 44% 100-150 nmol/L n = 54, 30% >150 nmol/L n = 25, 14%
Roy et al. (2007)	Women (n=78)	19-36 yrs	South Asian	Manchester, UK	19.72 ± 10.5 nmol/L	
Crowe, Steur, Allen, Appleby, Travis & Key, 2010)	Meat eaters (ME) n = 1388 Fish eaters (FE) n = 210 Vegetarians (V) n = 420 Vegans (Ve) n = 89	ME 55 ± 10 yrs FE 51 ± 12 yrs V 48 ± 13 yrs Ve 44 ± 14 yrs	Non-whites excluded (self-reported)	Oxfordshire, Buckinghamshire Manchester UK	ME = 77.0 (75.4 – 78.8)** FE = 72.2 (68.2 – 76.4)** V = 66.0 (63.3 – 68.8)** Ve = 55.8 (51.0-61.0)**	

Mahdy, Al-Emadi, Khanjar, Hammoudeh, Sarakbi, Siam & Abdelrahman (2010)	Healthy, healthcare volunteers N = 340 Males n = 136 Females n = 204	Males 40.8 yrs Females 35.9 yrs	N/A	Hamad Medical Corporation, Doha-Qatar	>75 nmol = 5% Males, 2% Females 50 – 75 nmol = 10.5% males, 8.9% females 25 – 50 nmol = 43.4% males, 25.1% females <25 nmol = 30% males, 38% females <7.5 nmol = 11% males, 26% females	
Itoh et al. (2011)	Males Fixed daytime n = 6 Rotating shift workers n = 4 Rotating shift workers with night work n = 4	 Day 51.8 ± 6.1 yrs Rotating 43.5 ± 4.8 yrs Rotating with nights 39.5 ± 4.7 yrs	N/A	Osaka, Japan	 Day = 26.0 (19.5-29.5) Rotating = 25.5 (23.5-26.8) Rotating with nights = 26.0 (23.5-27.8)	
National Diet and Nutrition Survey years 1, 2 and 3 combined (2012)	n = 3073	Girls (G)11-18 yrs Women (W) 19-64 yrs	UK population	UK	G = 42.5 ± 37.7nmol/L (20.4% <25nmol/L)	W = 49.6 ± 25.6nmol/L (18.6% <25nmol/L)

- Data presented mean ± standard deviation

** Data presented Confidence Intervals (CI)

The vitamin D content of the oily fish in the UK may not be similar to the oily fish consumed in Japan. Indeed, farmed fish will present lower amounts of 25 (OH) D than wild fish due to the diet consumed by such fish prior to being caught, i.e. pellets vs. phytoplankton (Holick, 2008). Similarly ethnicity cannot be determined by geographical area alone, yet has an extensive effect on 7-dehydroxycholesterol irradiation. As a result, evidence which suggests a significant proportion of a population or cohort may, or predisposed to, be 25 (OH) D deficient should be used with caution.

Four of the six studies in Table 3 are of similar latitude (London, 51.5^{O}N – Manchester, 53. 5^{O}N); as well as those of different latitudes such as Doha, Qatar (25.3^{O}N) and Osaka, Japan (34.7^{O}N). It has been estimated that a fair skinned person, with exposed face and forearms at mid-day in the UK would generate 2000 IU (50 µg) in 20-30 minutes, a similar amount would be achieved in a significantly shorter time period at a lower latitude i.e. Doha, Qatar. However, it has been hypothesised that those with skin pigmentation may require up to ten times as much exposure to synthesis a similar amount of previtamin D (Pearce & Cheetham, 2010). Indeed, the study by Roy et al. (2007) found a mean 25 (OH) D concentration of 19.72 ± 10.5 nmol/L, 94% of women of a South Asian descent had 25 (OH) D concentrations <37 nmol/L, 26% had concentrations <12.5 nmol/L. The study used a questionnaire to ascertain family origin as representation of participants' ethnicity. Van der Meer et al. (2006) previously observed, and controlled for, discrepancies between the self-reported ethnicity and ethnicity in relation to parentage. However, mixed family origins may confound assumed ethnicity by producing a different skin type than expected. As a result, as well as recording ethnicity the “Fitzpatrick Scale” (Fitzpatrick, 1988) has been used to determine the type of skin of participants in relation to UV response e.g. Type I = light, pale, white, always burns, never tans and Type VI = Black, very dark, Never burns, tans very easily, deeply pigmented. Nonetheless, the study by Roy et al. (2007) demonstrates why those with heavy skin pigmentation are an at-risk population, particularly in northern latitudes.

The effect of different dietary intakes on 25 (OH) D concentrations was explored in meat-eating, non-meat-fish-eating, vegetarian and vegan participants (Crowe, Steur, Allen, Appleby, Travis & Key, 2010). The study provided a cross-sectional analysis (n = 2107) of Caucasian (self-reported) males and females aged 20-76. The daily intakes of vitamin D were estimated using a semi-quantitative Food Frequency Questionnaire (FFQ). The FFQ required participants to recall food consumption over a period of time, which may cause memory recall bias or observer bias where participants over-estimate consumption of foods in question. However, due to the relatively small dispersion of vitamin D rich foods, FFQs have regularly been used in research. Moreover, as previously mentioned the vitamin D content of meat and vegetables may vary according to direct UVB exposure and in-direct UVB exposure i.e. in the food chain (Holick, 2008). Meat-eaters had a significantly higher ($p<0.001$) mean (range) intake of 3.1, 3.0-3.2 µg, than fish-eaters 2.2, 2.1-2.4 µg, vegetarians 1.2, 1.1-1.3 µg and vegans 0.7, 0.6-0.8 µg, which suggests avoidance of animal proteins may increase the risk of 25 (OH) D deficiency.

The study by Crowe et al. found no significant difference ($p = 0.140$) when it assessed the number of hours per week spent in outdoor activity in the summertime. The study acknowledged that time spent outdoors, without the time of day and amount of body surface area exposed, was no more than a proxy for UVB exposure. Moreover, the study of healthcare professionals in Qatar, latitude of 25.3^0N, found 87% of 340 healthy volunteers had a plasma 25 (OH) D concentration <50nmol/L (Mahdy, Al-Emadi, Khanjar, Hammoudeh, Sarakbi, Siam & Abdelrabman, 2009). 36.5% reported an average daily time spent outdoors >1 hour, 34.1% reported <0.5 hours a day and 29.4% reported no sun exposure. However, neither study assessed the working patterns i.e. shift patterns and environment i.e. inside or outside, of the participants, which may be a limiting factor.

Night work

According to the Office of National Statistics (2011) 14% of the UK's workforce engages with "shift work" most of the time, and 3.5% engage occasionally. The night shift may succeed the day or evening shift, depending on the individual shift cycle employed by the business. The night shift usually takes place between 11pm and 6am, and involves at least three hours between midnight and 5am (UK Government [UK GOV], 2013), taking the individual business shift cycle into consideration. UK GOV (2013) state that if one was to reject night shift working conditions, they are within their rights to do so without facing disciplinary action.

The different shift schedules represent the needs of employers from many different industries (Figure 1), which are directly influenced by the consumer demand for their product, and therefore represent a large degree of variation.

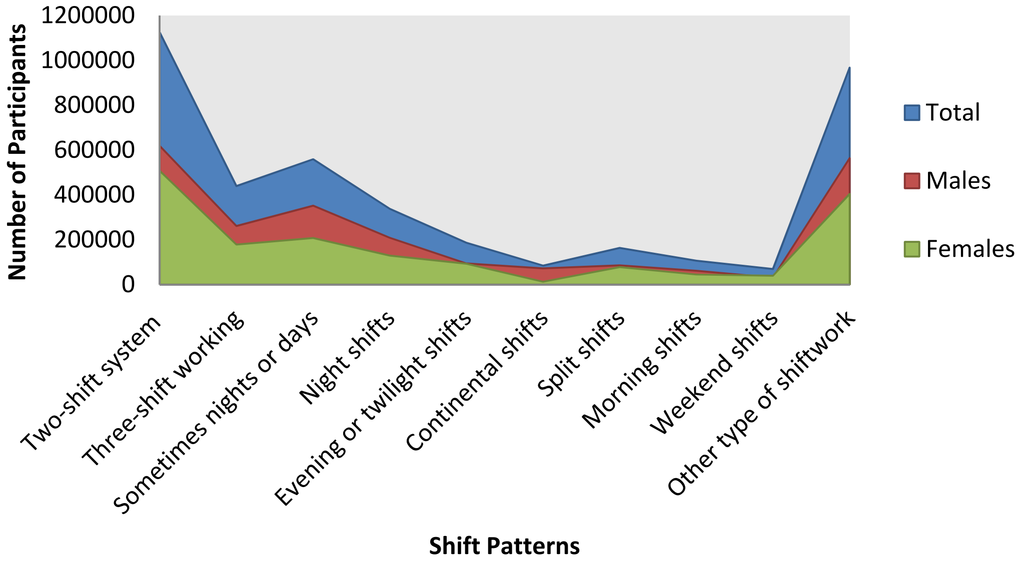

Figure 1: Common types of shift work in the UK (Office of National Statistics, 2011).

The most common shift schedule in Figure 1 was the two-shift system, which constituted a rotation of morning and evening shifts, with 617,895 men and 506,642 women working such hours. There were 337, 848 workers who worked night shifts full time, representing 8.3% of the sample. The three shift working schedule typically included one week of mornings, afternoons and evenings; overall 10.8% of the sample worked a three working shift. Additionally, it should be observed that 13.8% of the sample worked days as well as nights, however, data regarding the frequency of rotation was not available. Similarly, although the continental shift was relatively unpopular (2.1%) it did include some night work (following a three morning, two afternoons and two night shift pattern). Moreover, the evening/ twilight shifts incorporated 1500-2400 shifts. There was also a large proportion (23.9%) of undefined shift schedules which may also contribute to an overall measure of night shift engagement. Therefore participation and, thus, the effects of night shift work may be greater than initially thought based on the available data.

The gender differences in shift schedules may reflect the different socioeconomic roles of families, for example, a greater proportion (1.5% difference) of females worked evening or twilight shifts; which include part time shifts which would allow females to look after children in the day and work in the evening when partners or child carers are available. The data was compiled using a population based questionnaire, used to identify the most common worked shift pattern by individuals. However, the data lacks crucial information such as age, religion, gestation, combination of shift schedules and ability to work.

Vitamin D status in shift workers

Effect on health

As well as shift work in general, the role of nocturnal work within the shift cycle has been the subject of recent studies (Knutsson, 2003; Atkinson, Fullick, Grindey, Maclaren & Waterhouse, 2008; Lowden, Moreno, Holmbäck, Lennernäs & Tucker, 2010; PLoS Medicine Editors, 2011) specifically observing the association with cancer (Davis, Mirick & Stevens, 2001; Hansen, 2001; Schernhammer, Kroenke, Laden &

Hankinson, 2006; Kubo et al., 2006; Conlon, Lightfoot & Kreiger, 2007; Erren, Falaturl, Morfeld, Knauth, Reiter & Piekarski, 2010) and diabetes (Cheng et al., 2010).

It has been suggested that the decreased mobility associated with higher BMI levels restricts such individuals from gaining sufficient UVB exposure (Wortsman, Matsuoka, Chen, Lu & Holick, 2000; Blum et al., 2008). Suwazono et al. (2006) followed 4,328 Japanese male day (mean ± SD; 36.6 ± 10.9 years) and 2,926 (37.0 ± 9.4 years) alternating shift workers, of which night shifts were included, for 14 years. The study found the type of working pattern i.e. shift and day, was significantly associated with a ≥5% ($p = 0.001$; Odds ratio [OR] 1.14, 95% confidence intervals [CI] 1.06-1.23), ≥7.5% ($p = 0.012$; OR 1.13, 95% CI 1.03-1.24) and ≥10% ($p = 0.041$; OR 1.13, 95% CI 1.00-1.28) increase in BMI. Similarly, Biggi, Consonni, Galluzzo, Sogliani & Costa (2008) found significant associations with subcutaneous and visceral adipose tissue and vitamin D deficiency, to the extent that vitamin D deficiency was three times more evident in participants in the highest adipose tissue volume group. However, the study did not measure the level of UVB exposure, whether from artificial i.e. tanning bed, or natural light. Indeed, Arunabh, Pollack, Yeh and Aloia (2000) in healthy women (aged 20-80) with a mean (±SD) BMI of 23.9 ± 2.0 kg/m^2 found total body fat percentage (TBF), as derived by gold standard dual-energy X-ray absorptiometry, was independently associated with decreased 25 (OH) D levels ($p = 0.011$) alongside ethnicity ($p = 0.00001$), season ($p = 0.001$) and age ($p = 0.027$). The mean 25 (OH) D concentrations of the lowest TBF percentile group (<31%), 56.5 nmol/L, was significantly higher than that of the highest TBF percentile group (>42%), 44.2 nmol/L ($p<0.01$). However, the study (Arunabh et al., 2000) did not document the level of dispersion of Caucasian (n=239) and black (n=171) participants in TBF% groups, nor did it assess the working schedules or physical activity undertaken by participants. Nevertheless, literature suggests an inverse relationship exists between BMI and plasma 25 (OH) D concentrations (Giovannucci, Liu, Rimm, Hollis, Fuchs, Stampfer & Willett, 2006; Freedman et al., 2008; Scragg & Camargo, 2008).

Effect on Behaviour

There has been considerable emphasis on the lack of catering provision during night shifts, and the consequences of night shift workers being limited to high-sugar or high-fat foods, such as those typically found in a vending machine (Lennernäs, Hambraus & Akerstedt, 1994; Wong et al., 2010; Itoh et al., 2011). Indeed, many researchers report no distinct feeding time for night shift workers, which often results in continual feeding behaviours such as snacking and 'grazing' (Lowden, Moreno, Holmbäck, Lennernäs, & Tucker, 2010). However, any adaptations made by shift workers to their schedule are constantly undermined by holidays, sick days, days off, social events and weekends, where they revert to a social 'norm' (Atkinson, Fullick, Grindey, Maclaren & Waterhouse, 2008).

Effect on Dietary intake

A number of studies have emphasised the lack of catering provision during night shifts, consequently, night shift workers are drawn to high sugar or high fat foods, such as those typically in vending machines (Lennernäs, Hambraus & Akerstedt, 1994; Wong et al., 2010; Itoh et al., 2011). The few foods containing significant amounts of vitamin D_2 or D_3 i.e. oily fish, eggs, fortified milk and butter (Table 1), may be substituted for 'easier' to prepare options such as sweeter foods (Wong et al., 2010). Even so, due to disruption of circadian rhythms it has been reported that shift and permanent night workers suffer from gastrointestinal pain (Knutsson, 2003). Consequently, it was found nurses chose 'healthy foods', although not reported, to alleviate gastrointestinal pain (Persson & Marensson, 2006). The study by Persson and Marensson found nurses were aware of the tendency for night workers to gain weight and adjusted their diet to avoid such weight gain; an affect which may be explained by the level of education of the nurses and may not be apparent in those populations with a lower level of education.

The physical maladaption to altered circadian rhythms experienced by many shift and, particularly; night work has been identified as a leading cause of change to dietary intake (Atkinson, Fullick, Grindey, Maclaren & Waterhouse, 2008). In refuge collectors it was observed that morning workers (n = 42) consumed and expended more energy in the morning than afternoon (n = 56) and night shift workers (n = 34). No significant difference ($p > 0.01$) was observed in the mean (±SD) food intake between morning (3564 ± 104.5 kcal), afternoon (3619 ± 115 kcal) and night (3945 ± 177.7 kcal) shift patterns (de Assis, Kupek, Nahas & Bellisle, 2003). Night shift workers reported a high proportion of snacking during the night (10.2%) as did afternoon workers (9.6%); whereas, night workers reported a high proportion of snacking during dawn hours (10.9%) afternoon workers did not (1.1%). The higher proportion of snacking by night and afternoon workers, (44.4% and 44.9% respectively), may explain to an extent the lower levels of dietary intake of animal proteins, and potentially rich sources of vitamin D (Table 1). The consumption of animal proteins most commonly requires significant preparation and cooking time, which may be explain the higher intakes of convenience snacks such as pastries by afternoon and night workers. The increased propensity for the night workers to snack combined with reported higher mean (±SD) intake of fruit and vegetables (272 ± 16.5 kcal) than morning (110 ± 10.5 kcal) or afternoon (151 ± 12.3 kcal) workers may emphasise the potential avoidance of vitamin D rich dietary sources.

The relationship between 25 (OH) D levels and working patterns has been studied in Osaka, Japan, with 14 indoor male workers (Itoh, Weng, Saito, Ogawa, Nakayama, Hasegawa-Ohira, Morimoto, Maki & Takahashi, 2011). The six fixed daytime workers aged (mean ± SD) 51.8 ± 6.1 years were significantly ($p<0.02$) older than the four rotating shift workers without night shifts aged 43.5 ± 4.8 years and four rotating shift workers with night shifts aged 39.5 ± 4.7 years. The study found four participants, two fixed daytime workers, one from rotating shift work without night shifts and one from rotating shift work with night shifts, had 25-hydroxyvitamin D concentrations below the 50 nmol/L threshold for deficiency. The study concluded that night shift work was not associated with lower 25-hydroxyvitamin D concentrations, yet emphasised the need for larger studies including women to confirm the hypothesis. Nevertheless, the study's location, Osaka, Japan, was located at a latitude (34.7^{O}N) with considerable UVB exposure, more so than the UK where a greater latitude (53.5^{O}N) minimises UVB exposure. The average working time per month for fixed daytime workers was 160 ± 8.2 hours, 141 ± 24.6 hours for rotating shift workers without night shifts and 135 ± 15 hours for rotating shift workers with night shifts. Therefore, those workers participating in night shifts would potentially be able to spend more time outdoors and due to the shorter working times may have attenuated any deficiency during out of work hours, for example the study participants did not work weekends. However the study by Itoh et al., in an area with a higher level of oily fish consumption when compared to the UK and USA (Calvo, Whiting & Burton, 2004), did not assess the dietary intake of participants. Therefore, the study cannot determine whether night workers consumed more vitamin D rich foods to compensate for a lack of UVB exposure.

Current Policy

The current UK GOV (2013) policy does not require employers to disclose the potential risks associated with night shift work as a permanent, rotational or temporary working pattern. In 2009 a growing dossier of evidence claiming night shift work was "probably carcinogenic" lead to 38 Danish nurses, who had been diagnosed with breast cancer after working night shifts over a 20 year period, receiving compensation (Wise, 2009).

Conclusions

The previous literature has documented associations between nocturnal working schedules and adverse health effects. However, the extent to which permanent night shift work in the UK affects vitamin D status has not been elucidated. Indeed, those studies which include night shift work do not include permanent night workers, and do not reflect the vitamin D status of night and day workers from another UVB exposed area. Moreover, the literature does suggest shift workers, particularly night shift workers, have an altered dietary intake; however the subsequent effect on vitamin D intake has not been identified.

Research hypotheses;

Night shifts workers will have a significantly lower 25 (OH) D concentration than day shift workers.

Night shift workers will have a significantly lower dietary intake of vitamin D than day shift workers.

Null hypotheses;

There will be no significant difference in the 25 (OH) D concentrations of night and day shift workers.

There will be no significant difference in the dietary intakes of vitamin D of night and day shift workers.

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Research Paper

Vitamin D concentrations and dietary intake of night and day workers

Word count: 4000

Contents

Target Journal – Public Health Nutrition Journal (PHN)

Rationale – The PHN journal publishes research related to the promotion of good health and primary prevention of nutrition related illness in the population. PHN journal targets research on 'high-risk' populations linking to nutritional solutions for health promotion. PHN has published a number of UK based vitamin D research papers, one of which relates nurses' working schedules to vitamin D status. The current study would add to the current understanding of vitamin D and raise the awareness of the risk of deficiency. Therefore, PHN may be a potential choice for publication.

Abstract

Introduction: Vitamin D can be synthesised in the skin through exposure to ultraviolet blue (UVB) radiation and provides the majority of a human's daily intake. Night shift work, when compared to day shift work, may represent a reduced amount of UVB exposure and an altered dietary intake. The nocturnal working environment may adversely affect the dietary intake of vitamin D and calcium in shift workers. This study aims to investigate whether night shift workers have a lower vitamin D status than day time supermarket workers.

Design: 15 volunteers aged between 20-35 years were recruited from a UK 24 hour supermarket store. The participants provided a fasting fingerprick blood sample, completed height and weight assessments and a vitamin D and calcium FFQ.

Setting: A 24-hour supermarket in North Wales, UK.

Results: The intake of vitamin D was not significantly (t [13] = 0.226; p = 0.825) different between day workers (5.46 ± 2.83 µg/d) and night workers (5.16 ± 2.88 µg/d). The calcium intake of day workers (1712.42 ± 1207.87 mg/d) was not significantly different (t [13] = -0.154; p = 0.880) from night workers (1802.13 ± 1017.80 mg/d). The 25 (OH) D concentrations of day shift workers and night shift workers were not significantly different (t [13] = -0.290; p = 0.799).

Conclusions: The current study found no significant differences between the vitamin D statuses, and vitamin D and calcium dietary intakes, of night and day shift workers.

Introduction

There has been a wealth of data regarding vitamin D and associated health benefits, of which the role in calcium homeostasis has been considered the most important (Dusso, Brown & Slatopolsky, 2005; Chung et al., 2009). Vitamin D can be synthesised in the skin through exposure to ultraviolet blue (UVB) radiation, which provides the majority of a human's daily intake (Dusso, Brown & Slatopolsky, 2005). A small number of dietary sources exist, which contain vitamin D_3 or D_2, and contribute a small amount of an adequate daily intake.

Night shift work, when compared to day shift work, may represent a reduced amount of UVB exposure (Itoh et al., 2010). A number of studies have highlighted an altered dietary intake in shift workers (Lennernäs, Hambraus & Åkerstedt, 1994; Atkinson, Fullick, Grindley, Maclaren & Waterhouse, 2008; PLoS Medicine Editors, 2011), which may predispose nocturnal workers to an inadequate vitamin D intake (Itoh et al., 2011).

This study aims to investigate whether supermarket night shift workers have a lower vitamin D status, and dietary intake of vitamin D and calcium, than day time supermarket workers.

Experimental Methods

The study achieved ethical approval from the University's Ethics board prior to commencing assessments (Appendix 1). The researcher informed the University's Ethics board of all subsequent amendments made (Appendix 2). Five 24-hour supermarkets in North Wales and the North West of England, were contacted prior to the study (Appendix 3). The researcher arranged a formal meeting with each supermarket's representatives. The researcher explained the aims and rationale of the study to the supermarket representatives as well as the required space within the supermarket building. The supermarkets signed consent forms to confirm permission to use their facilities (Appendix 4). The researcher distributed posters within the supermarkets (Appendix 5). The researcher arranged two assessment days, one day and one night time, at the supermarket. The times of the assessment days were specific to the supermarket and the turnover time, where day and night workers were beginning their shift. Participants were asked to arrive at the assessment day after an eight hour fast.

Participants

The research study involved male and female supermarket workers aged between 20-35 years. Vitamin D concentration was used as the basis of a power calculation which suggested 26 participants per group, 52 in total, were required. Therefore, the study would aim to recruit a 20% larger sample size (n = 62) to counter for drop outs. The study used a volunteer sample where no financial incentive was used; participants received the results of their vitamin D feedback sheet (Appendix 6) in the post.

Participant eligibility criteria required them to be in full-time employment within either day working hours (08:30 – 21:30) or night working hours (21:30-08:30) for the previous three months (Appendix 7). Participants were given an information booklet prior to taking part, which outlined the study's procedure (Appendix 8). Participants signed a consent form (Appendix 9) and were screened for current pregnancies, or those in the last six months, and injuries to weight bearing joints i.e. hip knee or ankle, in the last six months (Appendix 10). Participants were also asked to self-report their ethnicity, their tanning bed use in the previous three months and medication (Appendix 9).

Assessments

The participants were initially asked to fulfil pre-assessment criteria questionnaires to ensure volunteers were eligible. A risk assessment was completed regarding the assessment location (Appendix 10). The height and then mass of participants was recorded, using a stadiometer and digital scales. Participants were asked to remove their shoes as well as any excessive, heavy, clothing for height and weight assessments. Once height and weight was recorded, the participant's Body Mass Index (BMI) was calculated using the equation in Figure 1.

$$BMI = \frac{mass\ (kg)}{[height\ x\ height(m)]}$$

Figure 1: BMI equation

The participants were then asked to sit down and to nominate a finger for a fingerprick blood sample (Appendix 11). The researcher wore clean gloves (GN99 Powder free Blue Nitrile Examination Gloves). The researcher explained that lancets used in the study were single-use and there was minimal risk of contamination. The researcher used an alcohol wipe (Isopropyl Alcohol 70% v/v, Sterets) to clean the nominate finger prior to, and immediately after, puncturing the skin on the nominated finger with the lancet (MD03330, Unistik 3 Extra, 2.0mm). The lancet was then disposed of in the sharps bin. The researcher positioned the bleeding finger over a 1.0 ml lithium heparin polypropylene tube. Once a sufficient sample had been achieved, the researcher used an alcohol wipe to clean the puncture site. The participant was given a cotton wool ball to maintain pressure on the puncture site. The participant was then asked to complete a 69-item vitamin D and calcium food frequency questionnaire (FFQ; Appendix 13), adapted from a previous study (Collins, O'Brien, Hill, Flynn, Cashman & Kiely, 2004). The participants were encouraged to ask any questions they had regarding the FFQ, such as portion size estimation and definitions of foods. The researcher ensured the FFQ's were fully completed by examination. If the FFQ required more information on any of the questions the researcher would either ask the participants to complete the questions fully or the researcher would ask relevant questions i.e. 'can you estimate how much you have?', and note down the answers. The FFQ did not identify different values for vitamin D_2 or D_3 due to a lack of such information in the nutrient composition tables (Crowe, Steur, Allen, Appleby, Travis & Key, 2010).

Laboratory Assay

Once all samples were collected from participants, an enzyme linked immunosorbent assay (ELISA; 25 hydroxyvitamin D ELISA Kit, 96-Well, Enzo Life Sciences, Lause, Switzerland) was used to determine plasma 25-hydroxyvitamin D (25 [OH] D) concentrations (Appendix 14). The blood samples were stored immediately below 5^0C and during transition to the laboratory. On arrival at the laboratory, plasma was separated by centrifugation and immediately frozen (-20^oC). Once all the samples had been collected and separated, they were thawed immediately prior to analysis. A pipette was used to transfer 90 µl of dissociation buffer into the wells followed by 10 µl of the standards and samples into the 96-well plate. Six standards used, alongside a non-specific binding (NSB) and a maximum binding (Bo) solution, which were duplicated horizontally. The samples were also duplicated horizontally in the 96-well plate. The solution was incubated at room temperature for one hour, and periodically hand shaken for five minutes every 15 minutes. After the wells were washed three times, pNPP solution was added followed by a stop solution. The wells were inserted into a plate reader which used an optical density of 405 nanometres (nm).

Analysis

The completed vitamin D and calcium FFQs were analysed using a computer software nutrient database called Microdiet. Microdiet utilised the 6th Edition of McCance and Widdowson's food composition database (McCance and Widdowson, 2002). The values for foods were multiplied by the frequency of consumption, such as once a day, once a month, ≥4 times a day etc..., and divided to produce an estimated daily value (Appendix 15). The results from the ELISA assay were inputted into a Microsoft Office Excel document (Appendix 16) which produced a linear relationship between the absorbencies elicited in the assay and concentrations provided by the company which produced the ELISA assay (Enzo Life Sciences, Lause, Switzerland). Subsequently, a trend analysis was used to provide concentrations for the absorbencies of the samples.

Statistical Analysis

The data was inputted to the SPSS software package (IBM, SPSS Statistics, Version 20) for Windows. Normal distribution of the variables was assessed using the Shapiro-Wilk's test, as the sample size was under 100. The homogeneity of the variance was also assessed for each variable. An independent *t* test was used to assess parametric data sets, whereas, the Mann Whitney U test was used for nonparametric data. A Bonferroni correction was used to adjust the level of significance as multiple tests for significance were used, P value below 0.17 was required for significant difference.

Results

One supermarket was able to take part in the study, three supermarkets were unable to take part within the study's timescale and one supermarket did not reply. The study included 17 participants, two participants chose to withdraw. The characteristics of participants' who did take part in presented in Table 1.

Table 1: Participant characteristics

Variable	Shift schedule	
	Day*	**Night***
Sample (n)	8 (*M* = 3; *F* = 5)	7 (*M* = 6; *F* = 1)
Age (years)	28.6 ± 5.8 (*M* = 22.3 ± 0.6; *F* = 32.4 ± 3.3)	22.6 ± 4.7 (*M* = 26.7 ± 4.6; *F* = 21)
Ethnicity	White, British	
BMI (kg/m^{-2})	28.5 ± 4.5 (*M* = 27.1 ± 4.5; *F* = 29.3 ± 4.7)	23.7 ± 1.1 (*M* = 23.9 ± 1.0; *F* = 22.3)

*Mean (±SD)

There were 15 participants in total who completed all the assessments, including eight day (three males and five females) and 7 night workers (six males and one female). The overall average age (mean ± SD) of the participants was 30.6 ± 15.8 years, 30.8 ± 4.8 years for day (males = 22.3 ± 0.6; Females = 32.4 ± 3.3 years) and 30.0 ± 1.4 years for night workers (males = 26.7 ± 4.6; Female = 21 years) respectively. The overall average age for males was 27.7 ± 4.2 years and 32.4 ± 3.3 years for females. The average height of the sample cohort was 168.8 ± 11.1 cm, males 175.3 ± 7.6 cm and females 159.0 ± 7.7 cm. Day workers had an average height of 165.6 ± 13.4 cm (males = 179.3 ± 8.1cm; females = 157.4 ±7.4cm) and night workers had an average height of 172.4 ± 7.1 cm (males = 173.3 ± 7.3 cm; female = 167 cm).

The average mass of participants was 74.4 ± 11.3 kg, males = 76.5 ± 8.8 kg; females = 71.2 ± 14.6 kg. Day workers had an average mass of 70.2 ± 4.7 kg (males = 86.4 ± 7.8 kg; females = 29.3 ± 4.7 kg) and night workers 78.0 ± 14.3 kg (males = 71.6 ± 3.4 kg; females = 62.2 kg). The average BMI of the study cohort was 26.2 ± 4.1 kg/m^2 (males = 24.9 ± 2.9 kg/m^2; females = 28.2 ± 5.1 kg/m^2). Day workers had an average BMI of 28.5 ± 4.5 kg/m^2 (males = 27.1 ± 4.5 kg/m^2; females = 29.3 ± 4.7 kg/m^2) and night workers 23.7 ± 1.1 kg/m^2 (males = 23.9 ± 1.0 kg/m^2; females = 22.3 kg/m^2).

Dietary intake

The total intake of vitamin D and calcium of participants can be observed in Table 2, as can the contribution to vitamin D and calcium intake by selected dietary sources. The overall intake of the study cohort of vitamin D was (mean ± SD) 5.3 ± 2.75 μg/d.

Table 2: Dietary intake of vitamin D and calcium by shift workers

	Day workers*	Night workers*	Overall*
Vitamin D intake (micrograms per day)	**5.46 ± 2.83 μg/d**	**5.16 ± 2.88 μg/d**	**5.3 ± 2.75 μg/d**
		T [13] = 0.226; P = 0.825	
Dairy**	0.38 ± 0.2 μg/d	0.93 ± 0.75 μg/d	0.64 ± 0.58 μg/d
Cereals	0.54 ± 0.44 μg/d	0.42 ± 0.46 μg/d	0.49 ± 0.44 μg/d
Meat***	1.33 ± 0.61 μg/d	1.07 ± 0.66 μg/d	1.21 ± 0.63 μg/d
Fish	2.24 ± 2.71 μg/d	2.69 ± 1.97 μg/d	2.45 ± 2.27 μg/d
Calcium intake (milligrams per day)	**1712.42 ± 1207.87 mg/d**	**1802.13 ± 1017.80 mg/d**	**1754.3 ± 1084.2 mg/d**
		T [13] = -0.154; P = 0.880	
Dairy**	629.04 ± 510. 79 mg/d	1222 ± 882.98 mg/d	905.75 ± 747.23 mg/d
Cereals	6.13 ± 5.73 mg/d	12.43 ± 15.27 mg/d	9.07 ± 11.27 mg/d
Meat***	74.46± 55.09 mg/d	52.15 ± 35.91 mg/d	64.05 ± 46.93 mg/d
Fish	32.52 ± 42.22 mg/d	18.85 ± 29.25 mg/d	26.14 ± 36.16 mg/d

*Mean ± SD

**Includes Milk, butter/spreads, cheese & yogurt

***Includes eggs

The intake of vitamin D was not significantly different (t [13] = 0.226; p = 0.825) between day workers (5.46 ± 2.83 µg/d) and night workers (5.16 ± 2.88 µg/d). The highest overall, and shift specific, intake of vitamin D was 2.45 ± 2.27 µg/d from fish dietary sources; night workers had a higher intake of vitamin D from fish than day workers (2.69 ± 1.97 µg/d vs. 2.24 ± 2.71 µg/d). The overall intake of vitamin D from dairy dietary sources was 0.64 ± 0.58 µg/d; day workers had the lowest shift specific intake of 0.38 ± 0.2 µg/d, whereas night workers had a higher shift specific intake of 0.93 ± 0.75 µg/d. The lowest overall vitamin D intake was 0.49 ± 0.44 µg/d from cereals (Day workers = 0.54 ± 0.44 µg/d; Night workers = 0.42 ± 0.46 µg/d). The second highest vitamin D intake was from meat dietary sources which contributed 1.21 ± 0.63 µg/d (Day workers 1.33 ± 0.61 µg/d; Night workers 1.07 ± 0.66 µg/d) to the overall daily vitamin D intake. Fish dietary sources contributed 2.45 ± 2.27 µg/d, (Day workers 2.24 ± 2.71 µg/d; Night workers 2.69 ± 1.97 µg/d) to the overall daily vitamin D intake.

The overall intake of calcium in the study cohort was 10754.3 ± 1084.2 mg/d. The calcium intake of day workers (1712.42 ± 1207.87 mg/d) and night workers (1802.13 ± 1017.80 mg/d) was not significantly different (t [13] = -0.154; p = 0.880). The highest intake of calcium was 905.75 ± 747.23 mg/d from dairy dietary sources (Day workers 629.04 ± 510. 79 mg/d; Night workers 1222 ± 882.98 mg/d). The lowest overall contribution of calcium from a dietary source was 9.07 ± 11.27 mg/d from cereals (Day workers = 6.13 ± 5.73 mg/d; Night workers = 12.43 ± 15.27 mg/d). Meat dietary sources contributed 64.05 ± 46.93 mg/d (Day workers = 74.46 ± 55.09 mg/d; Night workers = 52.15 ± 35.91 mg/d) to the overall calcium intake. Fish dietary sources contributed 26.14 ± 36.16 mg/d to the overall daily calcium intake, day workers had a higher calcium intake from fish than night workers (32.52 ± 42.22 mg/d vs. 18.85 ± 29.25 mg/d). There were four participants who recorded supplement use (Day workers = 3; Night workers = 1). Two participants used a vitamin D multivitamin, one night worker used a calcium supplement and one day worker used a cod liver oil supplement. One day worker used an evening primrose oil supplement and one day worker reported the use of loestrin 30 medication.

Plasma 25 (OH) D concentrations

The mean plasma 25 (OH) D concentrations for day and night shift workers can be observed in Figure 2.

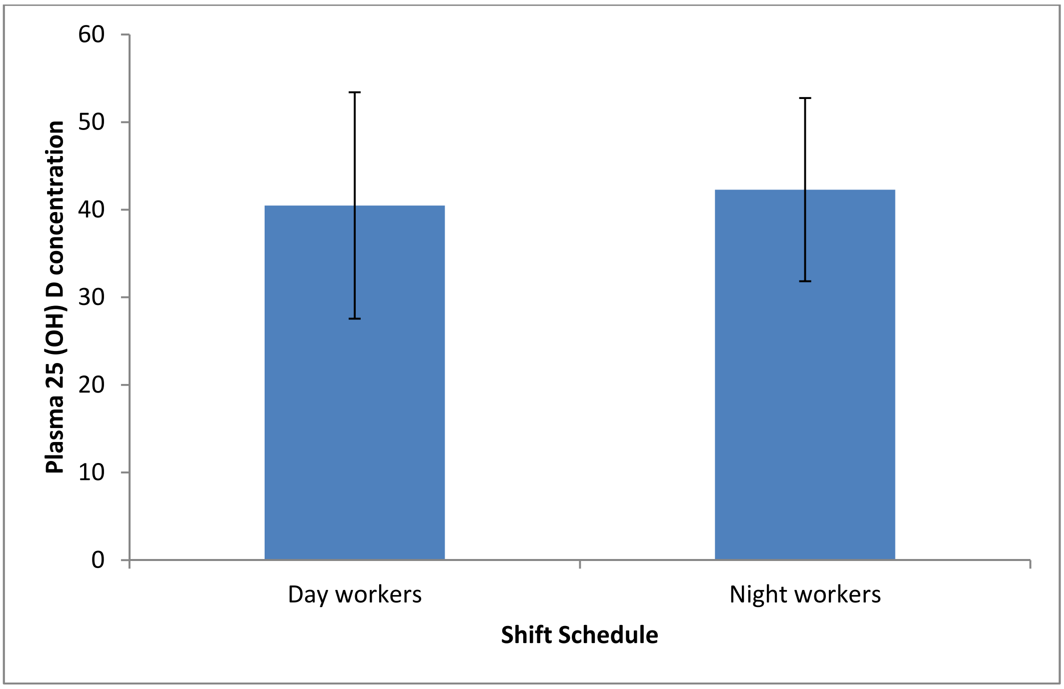

Figure 2: Mean plasma 25 (OH) D concentrations of day and night shift workers with standard deviations.

The overall average (mean ± SD) concentration of the study cohort was 41.44 ± 11.53 nmol/L. The day shift workers had a mean plasma 25 (OH) D concentration of 40.7 ± 13.06 nmol/L and night shift workers had an average concentration of 42.29 ± 10.461 nmol/L. The 25 (OH) D concentrations of day shift workers and night shift workers were not significantly different ($t [13] = -0.290$; $p = 0.799$). Overall there were two participants (13.3%) who had plasma 25 (OH) D concentrations below 30 nmol/L (Day workers = 12.5%; Night workers = 14.3%). 80.35% of the study cohort had 25 (OH) D concentrations below 50 nmol/L (Day workers = 75%; Night workers = 85.7%). No participants had a plasma 25 (OH) D concentration above 75 nmol/L, three participants had a concentration between 50-60 nmol/L (Day workers = 55.7 and 59.7 nmol/L; Night worker = 58.3 nmol/L).

Discussion

The current cross-sectional study investigated whether UK supermarket night shift workers had a lower plasma 25-hydroxyvitamin D (25 (OH) D) concentration, and a lower intake of vitamin D rich dietary sources than day workers. The study included men and women aged 20-35 years, however, found no significant ($p = 0.799$) difference in the plasma 25 (OH) D concentrations of night (mean 42.29 ± [SD] 10.461 nmol/L) and day workers (40.7 ± 13.06 nmol/L). All participants had a plasma 25 (OH) D concentration under the literatures' current level of vitamin D adequacy i.e. ≥75 nmol/l (Bischoff-Ferrari, Giovannuci, Willett, Dietrich & Dawson-Hughes, 2006; Holick & Chen, 2008). Moreover, two participants had plasma 25 (OH) D concentrations below 30 nmol/L, largely considered the clinical threshold for deficiency (Institute of Medicine, 2010; Barts and the London NHS Trust, 2011; NHS Mersey Cluster, 2012).

The current study found all participants had inadequate 25 (OH) D concentrations (<75 nmol/L) unlike the results published by Wallingford et al. (2013). Wallingford et al., based in Kingston Hospital, Canada, found a summer mean (±SD) serum 25 (OH) D concentration of 93.8 ± 42.2 nmol/L (73.9 ± 26.0 nmol/L in winter) in 70 young (35.8 ± 8.3 years) female nurses who worked rotating night and day shifts. Whereas a study in Japan (latitude 34.5^{O}N), using rotating shift workers with ($n = 4$ [RSN]) and without ($n = 3$ [RS]) night work, found slightly higher 25 (OH) D concentrations ([median, range] RSN = 64.9 nmol/L, 48.7 – 73.6 nmol/L; RS = 63.7 nmol/L, 58.7 – 66.9 nmol/L) to the current study. The influence of night shift work may be at its highest within the current study where participants wither worked day shifts or night shifts with no rotation

According to the Intersun: UV index (World Health Organisation, 2013), Kingston Hospital, Canada, typically receives a summer UV index score of between 5 and 7, whereas, Osaka Japan received a summer score between 8 and 10. The Japanese study should have elicited higher 25 (OH) D concentrations than the study by Wallingford et al. based on the latitude, and potential UVB exposure. However, the study by Wallingford et al. and Itoh et al. involved rotating shift workers with elements of night shift work. Further research demonstrated a subsequent rise in serum 25 (OH) D concentrations after summer months (Itoh, Mori, Matsumoto, Maki & Ogawa, 2011; Wallingford et al., 2013). However, the nurses', in the Wallingford et al. study, shift schedule (two 12 hour day shifts and two 12 hour night shifts followed by five days off) was different to the shift schedule that of the RSN and RS Japanese metal workers (Itoh et al., 2010). Indeed, the difference in shift schedule may have enabled the nurses to achieve adequate amount of UVB exposure on their days off. The potential difference in 25 (OH) D concentration caused by an increased or decreased latitude-dependent (excluding UV tanning beds) UVB exposure, as well as dietary differences, means the current study cannot be directly compared to the either study.

The previous literature regarding seasonal variation in vitamin D status, although limited in the number of UK specific investigations, suggests blood samples taken during the summer months would have a significantly higher 25 (OH) D concentration than blood samples taken in the winter months (Crowe, Steur, Allen, Appleby, Travis & Key, 2010). Crowe et al. used a cross-section of the UK population, which includes a large cultural diversity, and attempted to control for direct UVB exposure by measuring hours spent in outdoor activities per week, no significant differences were found (p = 0.140). However the study used a questionnaire, actual UVB exposure was not measured which may be subject to cloud cover, time spent in shade and sun protection used. Nevertheless, the previous literature would suggest the mean plasma 25 (OH) D concentrations in the current study would be lower if tested again during the winter months (October-March) when the UV index in the UK typically is 1-2 and UVB exposure is insufficient to synthesise vitamin D (WHO, 2012; Wallingford et al., 2013). A further decrease to the plasma 25 (OH) D concentrations of the supermarket workers would put them at-risk of deficiency (<30 nmol/L), increasing the risk of developing psoriasis, osteopenia and osteoporosis (Wallingford et al., 2013).

There is no previous literature regarding vitamin D intake in permanent night workers, however, the estimated intake of vitamin D and calcium of night workers in the current study is higher than non-supplemented estimates by other studies (Table 2; Review paper). The workplace environment i.e. supermarket, and working discipline of the study cohort i.e. general assistants, used in the study may have influenced the dietary intake of the participants. The employees of the supermarket involved benefitted from an employee discount card, which offered reduced food prices from the supermarket. As a consequence, the diet of night workers may not be affected by a lack of catering facilities as much as previously documented (Atkinson, Fullick, Grindey, Maclaren & Waterhouse, 2008), due to more opportunities to purchase food items. Indeed, supermarket workers present a different sample than other studies (Itoh et al., 2010; Wallingford et al., 2013), as they may buy ready to eat foods before they start their shift, during their lunch breaks and after their shift. This effect may, to an extent, elucidate why the present study found higher overall intakes of vitamin D (5.3 ± 2.75

µg/d) and calcium (1754.3 ± 1084.2 mg/d) when compared to the studies who used a similar assessment method (Table 2; Review paper).

There are no current dietary vitamin D intake recommendations for the UK population aged 4-65 (Department of Health [DoH], 1991). The dietary intakes presented in the current study satisfy the recommended intake (>5 ug/d) of other countries i.e. Ireland (53^{O}N), France (47^{O}N), Hungary (47.5^{O}N) and the WHO (Lanham-New et al., 2011). The DoH does, however, suggest groups 'at-risk' of low UVB exposure should consume at least 10 ug/d of vitamin D. These 'at-risk' populations consist of, children and adults aged 5-50 years, elderly people aged 50-70 years, people with dark skin and those with low UVB exposure to their skin (Lanham et al., 2011; DoH, 2012). The rate of vitamin D inadequacy in the current study may suggest that supermarket workers present a further 'at-risk' sub-population, particularly during the winter time. Indeed, the day workers in the current study had a lower vitamin D status than previous literature would have suggested (59. 2 ± 23.7 nmol/L; Macdonald et al., 2008). Nevertheless, the participants would benefit from a vitamin D supplementation, which may form part of a workplace health initiative as suggested by the previous research (PLoS Medicine Editors, 2011).

Limitations

The present study used a volunteer sample which limited the total sample size, as well as to those of a health conscious nature. The recent UVB exposure of participants was not assessed. Ethnicity was self-recorded which may have been miss-reported; for example although all participants reported to be Caucasian, there may have been subtle differences in skin pigmentation. BMI, although used in previous research (Adami et al., 2009; Itoh et al., 2010; Itoh et al., 2011; Wallingford et al., 2013), may have been confounded by higher levels of lean body mass.

Future Study

The current study was the first UK-based study to explore the effects of nocturnal working time on 25 (OH) D in a relatively young population (20-35 yrs), a further study may look to follow-up the current study to assess the consequential effects on bone mineral density. A similar study with an equal distribution of males and females, and ethnicity, would be able to identify whether pre-menopausal Caucasian or South Asian women are more ‘at-risk’ night shift work induced vitamin D deficiency than female day workers or age-matched males. Moreover, it would be pertinent to identify if particular working patterns and environments are more detrimental to vitamin D status than others. In order to improve reliability, a future study may include a repeated-measures design, where participants complete a FFQ, and provide a blood sample, twice with six months in between.

Conclusions

The current study found no significant differences between the vitamin D statuses, and vitamin D and calcium dietary intakes of, night and day shift workers. However, every participant had a plasma 25 (OH) D concentration under the proposed level of adequacy (<75 nmol/L) even though the estimated dietary intake was higher than intakes previously reported. Therefore, the study highlights the increased risk of vitamin D inadequacy of Caucasian supermarket workers aged 20-35 yrs.

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Appendices

Appendix 1 - University of Chester Ethical Approval letter

University of Chester

Faculty of Applied Sciences
Research Ethics Committee

frec@chester.ac.uk

Luke Davies
[redacted]

9th July 2013

Dear Luke,

Study title: **Vitamin D status and bone health in night and day shift workers.**
FREC reference: **817/13/LD/CSN**
Version number: **1**

Thank you for sending your application to the Faculty of Applied Sciences Research Ethics Committee for review.

I am pleased to confirm ethical approval for the above research, provided that you comply with the conditions set out in the attached document, and adhere to the processes described in your application form and supporting documentation.

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application Form	1	April 2013
Appendix 1 – List of References	1	April 2013
Appendix 2 – C.V. for Lead Researcher	1	April 2013
Appendix 4 – Letter of Invitation	1	April 2013
Appendix 5 – Participant Information Sheet	1	April 2013
Appendix 6 – Participant Consent Form	1	April 2013
Appendix 7 – Information Sheet	1	April 2013

FREC B
Approval letter – 2012/13

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Appendix 2 – Approved Amendments made to study

University of Chester

Faculty of Applied Sciences
Research Ethics Committee

frec@chester.ac.uk

Luke Davies
[redacted]

1st August 2013

Dear Luke,

Study title: **Vitamin D and bone health of shift workers**
FREC reference: **817/13/LD/CSN**
Version number: **1**

Thank you for providing notice of amendment to the above project.

The following omissions have been approved by the Faculty Research Ethics Committee:-

- The omission of the bone mineral density scan from the project.
- Removal of the details of the heel bone density scan from the Participant Information Sheet and the Gatekeeper invitation.
- Removal of Appendix 12 – GE Healthcare Sample Report from Achilles Insight.

With the Committee's best wishes for the success of this project.

Yours sincerely,

Dr. Stephen Fallows
Chair, Faculty Research Ethics Committee

FREC B
Approval of Research Amendments – 2012/13

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Appendix 3 – Letter to Supermarkets

Dear Whom it may concern,

My name is Luke Davies and I am a student at University of Chester studying for an MSc in Human Nutrition. I write to inform you of the current opportunity available to take part in a research project, examining the vitamin D status and bone health of day and night shift workers aged 20-35.

The study aims to identify whether night time workers are significantly more at risk of vitamin D deficiency and associated problems than day time workers.

What is required?

The participants would be asked to complete a questionnaire regarding their medical history and sign a consent form. The researcher will liaise with individuals identified as night or day shift workers to organise a 20-30 minute assessment prior to starting their shift. In order of the study to run effectively, it is necessary for the assessments to take place in the workplace. The study would require the use of a non-carpeted room, with nearby washing facilities, such as a sink with running water.

The researcher will measure height and weight and calculate body mass index. The participants will also be required to provide a fingerprick blood sample. Once the participants have completed the physical assessments they will be asked to complete a vitamin D food frequency questionnaire.

Yours sincerely

Mr Luke Davies, BSc.

Appendix 4 – Gatekeeper consent form

ASDA
Holt St
Wrexham
LL13 8HL

14th June 2013

Consent Form

Permission to use facilities

Title of Project: Vitamin D status and bone health in day and night shift workers

Name of Researcher: Luke Davies

Email:

I am conducting a research study into vitamin D levels in night and day shift workers as part of my MSc in Human Nutrition at the University of Chester. The study requires the use of facilities at the workplace, a non-carpeted room with cleaning facilities will be required. The equipment used in the study is completely portable and takes up minimal space. The participants will be asked to attend an assessment day prior to their shift, which should take no more than 20-30 minutes. The researcher will provide the scheduling of the study to the store manager. The researcher will contact the store manager in the event of alterations within the schedule.

I confirm that the above study has permission to use the required workplace facilities for the duration of the study.

____________________ ____________________ ________________

Name Date Signature

Appendix 5 – Poster

Vitamin D status and bone health in day and night shift workers

Please read

Why are you doing this study?

Vitamin D is essential to maintaining strong healthy bones. Vitamin D deficiency is a worldwide problem, even in developed countries. Night shift workers may be significantly more 'at-risk' of vitamin D deficiency, than day shift workers.

What would I have to do?

The study would require your participation in a 3-step assessment

1) Height and Weight
2) Fingerprick blood sample
3) Food Frequency Questionnaire

The assessment will take no more than 25 minutes, at your workplace, at a time and date of your choice .

What are the benefits of taking part?

Each participant will receive a result sheet with their vitamin D status.

If you would like to take part, or have any questions regarding the study please feel free to contact Luke Davies (lead researcher)

Email: [redacted]@chester.ac.uk

Appendix 6 – Vitamin D feedback sheet

University of Chester

Feedback

Vitamin D

Vitamin D is essential for healthy bones, and we get most of our vitamin D from exposure to sunlight. Everyone needs vitamin D to absorb calcium and phosphorus from their diet. These minerals are important for healthy bones. A lack of vitamin D i.e. vitamin D deficiency, can cause softening and weakening of bones and lead to bone deformities.

Vitamin D is relatively rare in foods, however, foods such as oily fish e.g. salmon, mackerel and sardines, are rich sources of vitamin D.

Source of vitamin D	Amount	Amount of vitamin D (microgramme)
Herring	85g	34.6 µg
Swordfish, cooked	85g	14.2 µg
Pink salmon, canned	85g	13.3 µg
Mackerel	85g	7.7 µg
Tuna, canned	85g	5 µg
Quaker Nutrition for Women Instant Oatmeal	1 packet	3.9 µg
Pork, spareribs, cooked	85g	2.2 µg
Liver, Beef cooked	99g	1.2 µg
Egg (vitamin D in yolk)	1 large	1.0 µg
Cheese, Swiss	28g	0.2 µg
Fortified Yogurt	170g	2.2 µg
Fortified Margarine	1 tablespoon	1.5 µg

Vitamin D levels	Adequacy level
>75 nmol/L	Optimal
50-70 nmol/L	Adequate
<50 nmol/L	Normal
<25 nmol/L	At risk of deficiency

Your vitamin D status	Current Adequacy

We can also create vitamin D in our bodies through exposure to sunlight. In fact, our body creates most of our vitamin D from direct sunlight. You should consult your doctor about how long you should exposure your skin for as it is different for everyone. You can find some useful information here; http://www.nhs.uk/Livewell/Summerhealth/Pages/vitamin-D-sunlight.aspx.

Appendix 7 – Participant invitation sheet

Dear Participant,

My name is Luke Davies, I am writing to invite you to participate in a research study concerning vitamin D status and dietary intake and bone mass.

To see if you are eligible please answer these questions;

1) Are you aged 20-35 years? YES/ NO

2) Are you currently in full time employment? YES/ NO

3) Are you available to take part in a research project that will involve some physical assessments and a food frequency questionnaire? YES/ NO

4) Do you work day shifts between the hours of 08:30 – 21:30? YES/ NO

5) Do you work night shifts between the hours of 21:30-08:30? YES/ NO

If you answer yes to the questions 1-3, and 4 or 5, you are eligible to participate in the study.

Please find some attached an information booklet, medical questionnaire and consent form.

If you are unsure of any of the questions or require more information please contact the researcher.

Mr. Luke Davies
Email:

Yours faithfully

Luke Davies

Appendix 8- Participant Information Booklet

University of Chester

QUI·DOCET·IN·DOCTRINA

Participant information sheet

Vitamin D status and bone health in night and day shift workers

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

The study aims to identify whether night time workers are significantly more at risk of vitamin D deficiency and associated problems than day time workers.

Why have I been chosen?

You have been chosen as a healthy participant, aged between 20-35, who has worked day or night shifts consecutively for a minimum of 3 months.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect you in any way.

What will happen to me if I take part?

The researcher will organise a time and date for you to attend an assessment day which suits your working pattern, where you will be asked to complete a 20-30 minute assessment prior to the start of your shift.

The researcher will ask you to complete a questionnaire explaining your medical history, which will indicate whether you are able to take part.

The researcher will measure your height, weight and Body Mass Index (BMI).

The researcher will take a fingerprick blood sample, depending on circulation another sample may be required. The blood sampling will take up to 5 minutes. Once you have completed these assessments, you will be asked to complete a food frequency questionnaire, which should take no more than 15 minutes.

What are the possible disadvantages and risks of taking part?

The fingerprick blood samples may cause some discomfort, however, this will disappear immediately afterwards.

What are the possible benefits of taking part?

By taking part, you will receive the results of the vitamin D assessment

What if something goes wrong?

If you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Professor Sarah Andrew, Dean of the Faculty of Applied Sciences, University of Chester, Parkgate Road, Chester, CH1 4BJ, 01244 513055.

Will my taking part in the study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential so that only the researcher carrying out the research will have access to such information.

What will happen to the results of the research study?

The results will be written up into a dissertation for my final project of my MSc. Individuals who participate will not be identified in any subsequent report or publication.

Who is organising the research?

The research is conducted as part of a MSc in Human Nutrition within the Department of Clinical and Nutrition Sciences at the University of Chester.

Who may I contact for further information?

If you would like more information about the research before you decide whether or not you would be willing to take part, please contact:

Luke Davies:

Thank you for your interest in this research.

Appendix 9- Consent form

University of Chester

QUI·DOCET·IN·DOCTRINA

Title of Project: Vitamin D status and bone health in day and night shift workers

Name of Researcher: Luke Davies

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected. ☐

3. I agree to take part in the above study. ☐

____________________	____________________	____________________
Name of Participant	Date	Signature

____________________	____________________	____________________
Researcher	Date	Signature

Appendix 10 – Health Screen

University of Chester

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Pre-test Questionnaire

Vitamin D status and bone of health in night and day shift workers.

Researcher *:* *Luke Davies*

Name:_________________________________ Test date:________________

Contact number:____________________________ Date of birth:___________

Email address ____

In order to ensure that this study is as safe and accurate as possible, it is important that each potential participant is screened for any factors that may influence the study. Please circle your answer to the following questions:

1. Are you pregnant, or have you been pregnant in the last six months? YES/NO

2. Have you injured your hip, knee or ankle joint in the last six months? YES/NO

Thank you for taking your time to fill in this form. If you have answered 'yes' to either of the above questions, unfortunately you will not be able to participate in this study.

Thank you for your time so far, these are additional questions specific to this research study. These questions aim to accurately assess your vitamin D levels and contributing factors.

Ethnicity

White, British	
White, Irish	
Other	
Mixed, Black Caribbean	
Black African	
White, Asian	

Asian, Indian	
British, Pakistani	
British, Bangladeshi	
British, African	
Chinese	

YES/NO

Have you used a 'tanning bed' in the previous 3 months?

Are you taking any other medication, other than that mentioned in the previous questions?

____

____

____

Do you have any injuries, past or present, concerning your heel, ankle or foot?

For example, metal plates, significant scarring or wound.

Appendix 11 – Risk assessment form

Name ………**Luke Davies**……………………………………..

Project title ………**Vitamin D status and bone health in day and night shift workers**……………………………….

Potential Hazard	Control Measures to be Adopted
Unintended lancet puncture	**The researcher will be responsible for the fingerprick blood sampling, and will only use a lancet when necessary, The lancets will be stored in a protective box to prevent unintended punctures. The lancets will be retractable. The lancets after use will be disposed of in a yellow sharps bin.**
Blood sample contamination	**Once the blood samples have been collected, they will be stored in a sealed ice box. The samples will be taken to the lab where they will be centrifuged, the plasma samples will be immediately frozen.**
Bruising at sample site	**The participant will be asked which hand they predominately use during their shift, the blood sample will be taken from the other hand. The sample site will be on the end of the participants fingers, off centre.**
Spillage	**The researcher will have necessary cleaning equipment ready for any spills. The location of the assessments will be in a non-carpeted room with cleaning/washing facilities.**
Drowsiness/ Light headedness	**The researcher will ensure the participant allows a short time period between providing the blood sample and starting the working shift. The participants will be able to sit down while they complete their vitamin D food frequency questionnaire.**
Fainting	**The researcher will ensure the participant is seated during and for a short time after the finger prick blood sampling. The participants will be given water if required.**

Appendix 12 – Fingerprick blood sampling protocol

<u>Finger prick blood sampling protocol</u>

1) Prepare and layout equipment; lancets, anticoagulant treated capillary tubes, sharps bin, alcohol wipes, non-latex gloves and spillage kit

- Put on non-latex gloves

2) If possible ask the participant to wash their hands with warm water

Ask the participant to nominate a finger to take the sample from

Massage the nominated finger to allow blood to come to the surface and hold the hand below waist level for 30 secs prior to sample

Wipe nominated finger with alcohol wipe

3) Remove lancet safety cap

Ensure the lancet is unused

4) Place lancet firmly on finger, positioned on the side of the finger tip if possible

Hold finger in place with one hand and position lancet with other hand

Press trigger of lancet fully until it clicks

Discard the lancet into a sharps bin

5) Observe puncture site, ensure lancet has punctured the skin

Maintaining a downward angle with the hand, massage blood flow towards the puncture site, without squeezing the finger

6) Wipe the first sample of blood to occur with an alcohol wipe

Place and maintain capillary tube at puncture site at a 45^{oC} to collect sample

-Depending on the rate of collection from the participant the protocol may be have to be repeated using another puncture site

7) Wipe the puncture site

Ensure the participant holds an alcohol wipe over the puncture site(s)

Ensure the samples are placed in a ice box immediately after sampling

Appendix 13 – Food Frequency Questionnaire

University of Chester

Food Frequency Questionnaire

Vitamin D and Calcium

These questions are designed to estimate the amount of vitamin D and calcium you get from the foods and drinks you usually consume. They are not to check if you have a healthy diet, or to criticise what you eat.

Thank you for taking the time to answer these questions carefully.

---

1. Do you drink glasses of milk (hot or cold)?

Yes	No	Non-dairy
☐	☐	☐

If yes, how often?

- Seldom ☐
- Once a month ☐
- 2-3 times/month ☐
- 1-2 times / week ☐
- 3-4 times / week ☐
- 5-6 times / week ☐
- Once a day ☐
- 2-3 times / day ☐
- ≥4 times / day ☐

2. Do you usually have milk in tea or coffee?

Yes	No
☐	☐

If yes, how often?

- Seldom ☐
- Once a month ☐
- 2-3 times/month ☐
- 1-2 times / week ☐
- 3-4 times / week ☐
- 5-6 times / week ☐
- Once a day ☐
- 2-3 times / day ☐
- ≥4 times / day ☐

What type of milk do you usually drink?

- Whole (full fat) ☐
- Semi-skimmed ☐
- Skimmed ☐
- Fortified ☐
- Soya ☐

3. Do you eat breakfast cereal with milk?

Yes	No
☐	☐

What type of milk do you usually drink?

- Whole (full fat) ☐
- Semi-skimmed ☐
- Skimmed ☐
- Fortified ☐
- Soya ☐

- Seldom ☐
- Once a month ☐
- 2-3 times/month ☐
- 1-2 times / week ☐
- 3-4 times / week ☐
- 5-6 times / week ☐
- Once a day ☐
- 2-3 times / day ☐
- ≥4 times / day ☐

How much milk do you typically add to cereal?
(estimate amount using different measures, for example ½ a mug)

____

____

____

4. If you usually eat breakfast cereal, what type of cereal do you eat?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
All Bran								
Weetabix/ Shredded Wheat								
Branflakes								
Special K								
Corn flakes/ Rice Krispies								
Other cereals (please state)								

5. Do you usually eat milk pudding, rice pudding or custard?

Yes ☐ No ☐

How often?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Milk pudding								
Rice pudding								
Custard								

---

6. Do you usually eat yogurt?

Yes ☐ No ☐

If yes, what type of yoghurt and how often would you have it?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Whole milk								
Fromage Frais								
Custard style yoghurt								
Drinking yoghurt/ Actimel								
Other yoghurt (please state)								

How much yoghurt would you usually eat each time?

1 regular pot (125g)

1 drinking yoghurt/actimel (100g)

1 large pot (500g)

7. Do you usually eat butter/spread on your bread?

Yes ☐ No ☐

If yes, what type of butter/spread and how often?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Butter								
Butter (70-80% fat)								
Olive oil-based spread								
Flora Original/ Light/ Other full/ low fat spread								
Other types of spread (please state)								

If yes, how much?

____
______

8. Do you usually eat cheese (all kinds)?

Yes	No
☐	☐

If yes, what type of cheese do you usually eat and how often?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Cheddar Cheese								
Cheddar type cheese, low fat								
Processed cheese (please state)								
Cheese spread (please state)								
Cottage Cheese								
Parmesan Cheese								
Other Cheese (please state)								

If yes, how much cheese do you have each time?

____

____

9. Do you usually eat cheese (all kinds) on pizza, on toast, on lasagne or with any kinds of foods?

Yes ☐ No ☐

If yes, how often?

Seldom ☐
Once a month ☐
2-3 times/month ☐
1-2 times / week ☐
3-4 times / week ☐
5-6 times / week ☐
Once a day ☐
2-3 times / day ☐
≥4 times / day ☐

10. Do you usually eat bread, wraps, and/or rolls?

Yes	No
☐	☐

If yes how often?

Seldom ☐
Once a month ☐
2-3 times/month ☐
1-2 times / week ☐
3-4 times / week ☐
5-6 times / week ☐
Once a day ☐
2-3 times / day ☐
≥4 times / day ☐

If yes, how many slices/pieces?

____

Which kind of toast/ bread/ rolls or crispbread do you usually eat?

White ☐
Brown ☐

____

11. Do you usually eat fish?

Yes	No
☐	☐

If yes, how often do you eat any of the following fish?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Fish fingers/deep-fried fish (please state which fish)								
Plaice/ Cod/ Haddock								
Salmon/ Trout								
Tinned salmon								
Mackerel								
Tuna in water/ oil								
Sardine in tomato/ oil								
Other fish (please state)								

If yes, how much fish do you eat each time?

____

12. Do you usually eat meat?

Yes ☐ No ☐

If yes, what type of meat do you usually eat and how often?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Sausages								
Pork								
Beef/ Steak								
Beef dishes								
Bacon								
Ham								
Lamb								
Liver								
Black pudding								
Chicken								
Chicken dishes								
Other meat (please state)								

If yes, how much do you usually eat each time? (please state if different quantities)

____

______

13. Do you usually eat eggs (cooked, in dishes e.g. omelette)?

If yes, how many eggs each time?

____

14. Do you usually eat cakes, chocolate and/or biscuits?

Yes	No
☐	☐

If yes, which of the following do you eat and how often?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Chocolate covered bar with fruit/ nuts/ wafer								
Milk chocolate								
Soft centred Chocolates								
Semi-sweet/ shortbread biscuits								
Biscuits, i.e. Mcvities								
Chocolate cake								
Sponge cake with/ without cream								
Madeira cake/ muffins								
Fruit cake								
Other cakes/ biscuits								
Other cakes, chocolate and/ or biscuits (please state)								

If yes, how much do you usually eat? (please state if different quantities)

____

____

15. Do you usually drink alcohol or carbonated drinks?

If yes, how often do you usually drink?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Lager/stout								
Red/ white wine								
Bitter								
Cider								
Coca Cola								
7-Up								
Lucozade								
Other drinks (please state)								

If yes, how much do you usually drink?

____

____

______

16. a) Do you usually consume beverages fortified with vitamin D and/ or calcium?

Yes	No
☐	☐

If yes, how often do you consume the following?

	Seldom	Once a month	2-3 times / month	1-2 times / week	3-4 times / week	5-6 times / week	Once a day	≥4 times / day
Fortified squeeze orange juice								
Fortified Tropicana orange juice								
Other fortified orange juice								
Other drinks fortified with vitamin D and/ or calcium								
Other drinks with vitamin D and/ or calcium (please state)								

If yes, how much do you usually consume?

____

____
______

16 b) Please name any foods that you consume that are fortified with vitamin D and/ or calcium that we have not covered so far?

____

____
______

17. Do you take dietary supplements (vitamin/ mineral tablets, cod liver oil, fish oil, herbal or other 'natural' medicine)?

Yes	No
☐	☐

Name of Product	Frequency (dose/ day or week)	Duration of use

Please tick if the respondent is taking vitamin D and/ or calcium supplements daily.
(Researcher will tick this)

1. Vitamin D (5 mg)
2. Vitamin D (10 mg)
3. Vitamin D (15 mg)
4. Vitamin D (>20 mg)
5. Calcium (250-500mg)
6. Calcium (501-1000mg)
7. Calcium (>1000mg)